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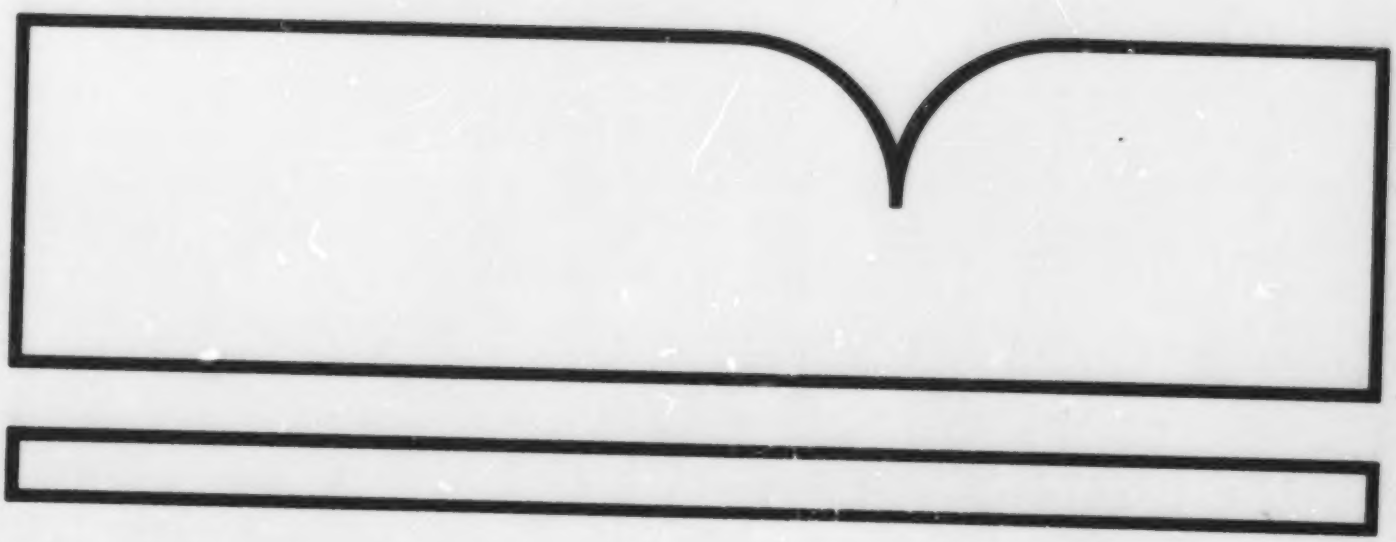

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Relative Sensitivity of Six Estuarine Fishes to Carbophenothion, Chlorpyrifos, and Fenvalerate[1]

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The acute toxicity (96–hr LC_{50}) of carbophenothion, chlorpyrifos, and fenvalerate to six estuarine fishes was determined in flow-through laboratory tests. The atherinid fishes (*Menidia menidia, M. peninsulae, M. beryllina,* and *Leuresthes tenuis*) consistently were among the most sensitive species tested and were similar to each other in their sensitivity to pesticides. The sensitivity of sheepshead minnows (*Cyprinodon variegatus*) to carbophenothion was the same as that of the atherinids. For fenvalerate, the sheepshead minnow LC_{50} was an order of magnitude greater than that of the most sensitive atherinid, whereas the LC_{50} for chlorpyrifos and sheepshead minnows was two orders of magnitude greater. Gulf toadfish (*Opsanus beta*) were the least sensitive fish tested with carbophenothion and chlorpyrifos and their 96–hr LC_{50} for fenvalerate ranked between the LC_{50} for sheepshead minnows and atherinids. Test results were compared to acute toxicity data for other estuarine fishes and invertebrates. © 1985 Academic Press, Inc.

INTRODUCTION

Information on the relative sensitivity of estuarine fishes to pesticide poisoning commonly is limited to those few species that have been developed for routine testing. From the greater variety of freshwater fishes that have been tested, several researchers have shown that species from the same family generally are more similar in sensitivity to a compound than species of different families. Macek and McAllister (1970) tested nine pesticides and 12 freshwater fishes from five families. Pickering *et al.* (1962) tested three centrarchid species against three organophosphate pesticides. Both studies reported homogeneity in sensitivity of species within a family, with differences among median effect concentrations generally less than a factor of 6. LeBlanc (1984) also reported similarities of pesticide toxicity within families of fishes. By calculating ratios of high-to-low values for acute LC_{50}s of metals and organochlorine pesticides from 17 water-quality criteria documents, Stephan (1982) found that significant variability was caused by differences in sensitivity among species within families as well as differences among families and classes of fishes.

We tested four species from the family Atherinidae: Atlantic silverside, *Menidia menidia;* tidewater silverside, *M. peninsulae;* inland silverside, *M. beryllina;* and California grunion, *Leuresthes tenuis.* The Atlantic silverside is one of the most abundant estuarine fishes of the Atlantic seaboard (DeSylva *et al.*, 1962; Dahlberg, 1972; Cain and Dean, 1976) and is forage for important commercial and recreational species (Bigelow and Schroeder, 1953; Middaugh, 1981). Atlantic silversides inhabit salt and

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brackish water from the Magdalen Islands, Canada, to northern Florida (Cox, 1921; Gosline, 1948; Johnson, 1975). Tidewater silversides are abundant in coastal waters of 15‰ salinity or higher (Johnson, 1975; Echelle and Mosier, 1982; Lucas, 1982; Middaugh and Hemmer, 1984) and are found from northeastern Florida to Mississippi and from Galveston Bay, Texas, to Tamiahua, Veracruz, Mexico (Chernoff *et al.*, 1981). Inland silversides, *M. beryllina* (syn. *M. audens*, Chernoff *et al.*, 1981) are the most widely distributed silverside that we tested. They occur in estuaries from Massachusetts to Texas and are found in freshwater rivers, reservoirs, and lakes in Florida, Mississippi, Louisiana, Texas, Arkansas, Oklahoma, Tennessee, and California (Robbins, 1969; Cook and Moore, 1970; Chernoff *et al.*, 1981). While euryhaline, inland silversides occur most frequently in freshwater or in waters with salinity that ranges to 19‰ (Echelle and Mosier, 1982; Middaugh *et al.*, in press) and are important as forage. California grunion support a recreational fishery along southern California beaches during spawning runs (Clark, 1925; Walker, 1949, 1952). Grunion are found in nearshore coastal waters from Bahia Magdelena on the outer coast of Baja California Sur northward to Monterey Bay, California (Moffatt and Thomson, 1975). They commonly inhabit coastal or estuarine areas where salinity ranges from 35 to 40‰ and temperature ranges from 12 to 32°C (Reynolds and Thomson, 1974).

We also tested sheepshead minnows (*Cyprinodon variegatus*), Family Cyprinodontidae, and Gulf toadfish (*Opsanus beta*), family Batrachoididae. These fishes are omnivorous and primarily associated with benthic habitats. Sheepshead minnows inhabit shallow bays with brackish water and are distributed along the Atlantic and Gulf coasts of the United States to Venezuela and throughout the West Indies (Hildebrand and Schroeder, 1972; Hoese and Moore, 1977). Gulf toadfish inhabit coastal waters throughout the West Indies and from Cape Sable, Florida, to Yucatan, Mexico, and are associated with reefs and jetties (Hoese and Moore, 1977). We have developed methods to culture and test these two species and the atherinids in our laboratory through partial or complete life cycles (Goodman *et al.*, 1985a,b; Hansen and Parrish, 1977).

The chemicals chosen for our tests are applied to control a variety of pests on crops, ornamentals, and livestock (Farm Chemicals Handbook, 1984) and could enter estuaries as a result of drift during application or in surface runoff following application for agricultural crop pest control. Carbophenothion (*S*-[(p-chlorophenylthio) methyl]0,0-diethyl phosphorodithioate) and chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl)-phosphorothioate] were selected as test materials because they represent two organophosphate compounds with different degrees of chlorination. Fenvalerate [cyano(3 phenoxyphenyl) methyl 4-chloroalpha-(1-methylethyl) benzeneacetate] was tested because it is among the most widely used synthetic pyrethroids.

The objectives of our study were to determine the acute toxicity of three structurally different insecticides to six species of estuarine fishes and to compare the sensitivity of the fishes. We used six species that can be reared in the laboratory for acute or partial life cycle testing and three chemicals that represent contemporary technology in chemical pest control. A limited toxicological data set such as this can serve as a reference for placing individual or incidental data into perspective (LeBlanc, 1984).

MATERIALS AND METHODS

All fish tested were juveniles less than 80 days posthatch. Age varied with species depending upon available culture space and other testing schedules. Silversides and

sheepshead minnows were spawned and reared using laboratory procedures described by Goodman *et al.* (1985b), Middaugh and Hemmer (1984), and Hansen (1978). California grunion were collected near Blacks Beach, LaJolla, California, as spawned embryos and shipped to the Gulf Breeze Environmental Research Laboratory for hatching and rearing (Borthwick *et al.*, 1985; Goodman *et al.*, 1985a). Gulf toadfish embryos or larvae attached to a spawning substrate (rocks or tiles) were collected by divers from Santa Rosa Sound near the Gulf Breeze Laboratory. Spawning substrates were immersed in aquaria with flowing seawater and hatched fish were fed *Artemia* daily until used in testing.

All tests were conducted according to ASTM guidelines for acute toxicity tests (ASTM, 1984). For flow-through toxicity tests, we used 60-liter glass aquaria supplied with 15 or 20 liters filtered (20 μm) seawater per hour; a self-starting overflow siphon provided a 20-liter draw-down over 15 min. In order to facilitate observation and counting, fish were confined to 3.8-liter (1-gal) glass jars fitted with two screened (140-μm mesh nylon screen) openings, 3 cm diameter, 1 cm above the base. Each test aquarium contained two jars, 10 fish per jar; the overflow siphon ensured exchange of test mixture in the jar. Gulf toadfish were removed from spawning substrates before testing.

Stock solutions for all tests were prepared in solvent carriers (acetone or triethylene glycol) from technical grade pesticide obtained commercially or from the manufacturer. A syringe pump metered 10 ml of toxicant solution per day into the stream of incoming seawater, where it was mixed and delivered to the test aquaria. A control and carrier control were maintained along with five exposure concentrations of each pesticide. Carrier concentrations were constant for all exposures within a test; 28 μl/liter was used for tests conducted at 15 liters/hr flow rates and 20 μl/liter for 20 liters/hr flow rates. Temperature was maintained by immersing the aquaria in a water bath; salinity varied with incoming seawater. Salinity for the inland silverside test with chlorpyrifos was adjusted to 5‰ by diluting filtered seawater with dechlorinated tap water. Temperature and salinity were monitored continuously; dissolved oxygen and pH were measured at 48 and 96 hr.

Static-renewal tests were conducted with Gulf toadfish in order to reduce carbophenothion and chlorpyrifos discharges to our wastewater treatment system. ASTM guidelines were followed (ASTM, 1984). Tests were conducted in 3.8-liter glass jars with five fish per jar (10 fish per concentration). Temperature was monitored continuously; pH, salinity, and dissolved oxygen were measured daily. Test solutions were changed daily.

Exposure concentrations were measured at 48 and 96 hr during each test. Mortality was recorded daily and dead animals were removed when discovered. The 96-hr LC_{50}s were calculated by probit analyses (Finney, 1971) or the moving average procedure (Kendall and Stuart, 1973).

Test concentrations were analyzed from 1-liter water samples extracted twice with 100 ml of nanograde petroleum ether (Burdick Jackson Laboratories, Muskegon, Mich.) by shaking for 1 min in a 2-liter separatory funnel. Solvent extracts were dried by passage through heat-treated (600°C) glass wool and concentrated to approximately 20 ml in a Kuderna–Danish concentrator fitted with a Snyder column. Concentration to 20 ml was conducted in a steam cabinet, and the extract concentrated to 2 ml under nitrogen at 35°C. Quantitation of chlorpyrifos and carbophenothion was performed with a Hewlett-Packard Model 5730A gas chromatograph equipped with a dual N/P

flame ionization detector; fenvalerate was quantitated on a Hewlett-Packard Model 5710 gas chromatograph with a ^{63}Ni electron-capture detector. All operating conditions for gas chromatographs were the same as those described by Schimmel *et al.* (1983).

RESULTS

Carbophenothion

Carbophenothion was most toxic to California grunion; the 96-hr LC_{50} was 2.3 μg/liter (Table 1). Tests with other atherinids yielded LC_{50}s that ranged from 7.7 to 9.5 μg/liter. For sheepshead minnows, the LC_{50} was 4.4 μg/liter. Gulf toadfish were so acutely insensitive to carbophenothion that exposure up to 1400 μg/liter for 96 hr produced 20% or less mortality. However, at concentrations of 170 μg/liter and greater, toadfish became lethargic and less responsive to prodding than control animals. The ratio of high-to-low 96-hr LC_{50}s for carbophenothion among the six species tested exceeded 600. For atherinids the high-to-low ratio was 4.1.

Chlorpyrifos

Toadfish also were acutely insensitive to chlorpyrifos; the 96-hr LC_{50} was 520 μg/liter (Table 1). The LC_{50} for sheepshead minnows was 136 μg/liter, whereas LC_{50}s for atherinids ranged from 1.3 μg/liter for *M. peninsulae* and *L. tenuis* to 4.2 μg/liter for *M. beryllina*. The high-to-low ratio for species exposed to chlorpyrifos was 400. Among atherinids the ratio was 3.2.

Although differences in test temperature varied by 7°C, we believe that these differences did not bias our results. For example, the highest LC_{50} for chlorpyrifos was for sheepshead minnows and this species was tested at the highest temperature. Salinity differences also are considered insignificant since, in an additional test, the 96-hr LC_{50} for chlorpyrifos to inland silversides was 4.0 μg/liter at 24‰ compared to 4.2 μg/liter at 5‰.

Fenvalerate

LC_{50}s for atherinids tested with fenvalerate were 0.3 μg/liter for *M. menidia* and *L. tenuis* and 1.0 μg/liter for *M. peninsulae* and *M. beryllina* (Table 1). These data yielded a high-to-low ratio of 3.3. The 96-hr LC_{50} for Gulf toadfish was 2.4 μg/liter, whereas sheepshead minnows were least sensitive with a LC_{50} of 5.0 μg/liter. The combined data set produced a high-to-low ratio of 16.

DISCUSSION

Although there were statistically significant differences in LC_{50}s among species, comparative toxicological assessments should be based on interpretations of results of more than a single test. The breadth of confidence limits for a time-dependent LC_{50} depends upon the distribution of deaths over the exposure concentrations and gradation of exposure concentrations, and is pertinent to results of an individual test only (Stephan, 1977). Acute LC_{50}s obtained from repeated tests with a single species-toxicant combination within a laboratory commonly fall within a factor of 2, while the same species-toxicant LC_{50}s generated among laboratories that employ standard test procedures usually fall within a factor of 4 (Schimmel, 1981).

TABLE 1

ACUTE TOXICITY OF CARBOPHENOTHION, CHLORPYRIFOS, AND FENVALERATE TO SIX SPECIES OF ESTAURINE FISHES

Pesticide	Species	Age (days)	96-hr LC_{50}, μg/liter[a] (95% CI)	Temp., °C mean (range)	Salinity ‰ mean (range)
Carbophenothion	*Cyprinodon variegatus*	Juv.	4.4 (3.9–5.0)	26.3 (25.5–27.0)	21.6 (21.2–22.0)
	Menidia menidia	36	7.7 (5.7–12.3)	24.9 (23.0–26.0)	25.5 (24.0–26.5)
	Menidia peninsulae	60	9.0 (6.8–15.4)	25.0 (24.0–26.0)	22.0 (22.0–22.0)
	Menidia beryllina	18	9.5 (7.7–10.3)	24.0 (23.0–24.5)	20.3 (19.0–22.0)
	Leuresthes tenuis	20	2.3 (1.6–2.6)	25.5 (25.0–26.0)	22.5 (22.0–23.0)
	Opsanus beta	80	>1400[b]	26.0 (26.0–26.0)	26.6 (26.5–27.0)
Chlorpyrifos	*C. variegatus*	35	136 (113–153)[c]	31.4 (30.5–32.0)	10.3 (7.0–19.0)
	M. menidia	53	1.7 (1.4–2.0)[c]	27.5 (22.0–28.0)	24.3 (24.0–25.0)
	M. peninsulae	60	1.3 (1.0–1.7)	24.6 (24.5–25.0)	19.3 (18.0–20.5)
	M. beryllina	72	4.2 (3.4–5.4)	24.5 (24.0–25.0)	5.0 (5.0–5.0)
	L. tenuis	28	1.3 (1.0–1.7)[d]	27.1 (27.0–27.3)	30.9 (29.5–32.0)
	O. beta	Juv.	520 (450–600)[b,e]	30.0 (29.0–30.0)	25.0 (22.5–26.5)
Fenvalerate	*C. variegatus*	10	5.0 (4.8–5.3)[c]	30.0 (29.0–30.0)	26.5 (25.0–28.0)
	M. menidia	42	0.3 (0.21–0.41)[c]	24.1 (23.5–25.0)	25.0 (25.0–25.0)
	M. peninsulae	70	1.0 (0.95–1.1)[f]	24.5 (23.5–25.5)	27.1 (26.0–28.0)
	M. beryllina	25	1.0 (0.86–1.3)	24.0 (22.5–24.5)	20.4 (19.0–22.0)
	L. tenuis	31	0.3 (0.21–0.37)	25.9 (25.5–26.3)	24.9 (22.5–27.3)
	O. beta	33	2.4 (2.3–3.3)	28.2 (26.0–30.0)	25.8 (24.5–27.0)

[a] Flow-through tests, except as noted; LC_{50} based on measured concentrations and probit analyses, except as noted.
[b] Static-renewal test; LC_{50} based on measured concentrations and probit analyses.
[c] From Schimmel *et al.*, 1983.
[d] From Borthwick *et al.*, 1985.
[e] Personal communication, D. J. Hansen, U.S. EPA, Narragansett, R.I.
[f] LC_{50} calculated by moving average procedure.

Relative Sensitivity

No species consistently yielded the lowest LC_{50}s nor were relative differences among family representatives consistent for all three pesticides. The atherinids were similar in their sensitivity to the pesticides. Borthwick *et al.* (1985) found comparable results for three atherinids exposed to thiobencarb, a carbamate herbicide. Sheepshead minnows, perhaps the estuarine fish most commonly tested, have been considered insensitive because of their ability to survive extreme ranges in temperature, salinity, and dissolved oxygen. However, their toxicological sensitivity places them among the most sensitive fish species exposed to carbophenothion and among the most resistant to chlorpyrifos or fenvalerate. The sheepshead minnow LC_{50} was approximately 100× that for atherinids tested with chlorpyrifos: the ratio was 5–7× for fenvalerate and the LC_{50}s were equal for carbophenothion. The ratio between LC_{50}s for sheepshead minnows and atherinids was 2–7× for thiobencarb; 1370/204–817 (Schimmel *et al.*, 1983; Borthwick *et al.*, 1985) and 4× for permethrin; 7.8/2.2 (Schimmel *et al.*, 1983). Roberts *et al.* (1982) found a 2–3× range in relative sensitivity of sheepshead minnows and Atlantic silversides for sodium lauryl sulfate, cadmium, and methomyl in static tests. In our tests, Gulf toadfish were consistently less sensitive than atherinids—by a factor of 2–8× for fenvalerate, 125–400× for chlorpyrifos, and >150× for carbophenothion.

Comparative Toxicity

We were able to find only a limited database on the acute toxicity of carbophenothion, chlorpyrifos, and fenvalerate to estuarine animals (Table 2). The additional LC_{50}s did not extend the range of least and most sensitive responses for any one species beyond a factor of 4, or expand the range of fish LC_{50}s appreciably beyond our highs and lows. However, the chlorpyrifos 96-hr LC_{50} of 0.58 μg/liter to striped bass, *Morone saxatilis* (Korn and Earnest, 1974) was approximately one-half that for the most sensitive species tested.

The range of LC_{50}s reported for marine crustaceans and the pesticides tested encompasses up to 2 orders of magnitude, and thus is similar to the range of responses observed for fishes. For carbophenothion, only one crustacean LC_{50} was less than that of the most sensitive fish. For chlorpyrifos, the difference in LC_{50}s between the most sensitive fish, *M. saxatilis*, and the most sensitive crustacean, *Mysidopsis bahia*, was greater than an order of magnitude (LC_{50}s of 0.58 μg/liter and 0.035 μg/liter, respectively). The lowest atherinid LC_{50} for fenvalerate (0.3 μg/liter) was 37× higher than that for *M. bahia* (0.008 μg/liter). These differences agree with those for freshwater organisms reported by LeBlanc (1984), for which organophosphate and carbamate pesticides were orders of magnitude more toxic to waterflea, *Daphnia magna*, than bluegill, *Lepomis macrochirus*.

For estuarine toxicity testing programs, sheepshead minnows are easy to culture and maintain in a laboratory, whereas techniques for rearing and handling atherinids have been developed only recently. Information on the relative sensitivity of atherinids and sheepshead minnows to pesticides may determine whether data from a single species is useful for environmental risk assessments. From our results, sheepshead minnows differed in varying degrees from atherinids in their acute sensitivity to carbophenothion, chlorpyrifos, and fenvalerate. The atherinids were consistently among the most sensitive estuarine fishes tested and, therefore, would be appropriate additions to testing programs designed to provide acute toxicity data for use in risk assessment

TABLE 2

LC_{50}s (μg/liter, 96-hr) FOR CARBOPHENOTHION, CHLORPYRIFOS, AND FENVALERATE TESTED WITH ESTAURINE FISHES AND INVERTEBRATES

	Carbophenothion	Chlorpyrifos	Fenvalerate
Fish species			
Cyprinodon variegatus	4.4[a], 2.8[f]	136[b]	5.0[b]
Menidia menidia	7.7[a]	1.7[b], 0.5–3.0[i]	0.3[b]
M. peninsulae	9.0[a]	1.3[a], 0.4–1.3[i]	1.0[a]
M. beryllina	9.5[a]	4.2[a]	1.0[a]
Leuresthes tenuis	2.3[a]	1.0–1.3[i]	0.3[a]
Opsanus beta	>1400[a] (static)	520[c]	2.4[a], 5.4[b]
Lagodon rhomboides	7.7[f]		
Leiostomus xanthurus	>206[f]		
Mugil cephalus		5.4[b]	0.58[b]
Fundulus heteroclitus		4.7[d]	
Morone saxatilis		0.58[e]	
Alburnus alburnus			2–3[g] (static)
Salmo salar			1.2[h] (static)
Invertebrate species			
Homarus americanus			0.14[h] (static)
Crangon septemspinosa			0.04[h] (static)
Mysidopsis bahia	3.0[f]	0.035[b]	0.008[b]
Penaeus duorarum	0.47[f]		0.84[b]
Palaemonetes pugio	2.0–4.6[f]		<0.003[j]

[a] This study.
[b] Schimmel *et al.*, 1983.
[c] D. J. Hansen, U.S. EPA, Narragansett, RI.
[d] Thirugnanam and Forgash, 1977.
[e] Korn and Earnest, 1974.
[f] U.S. EPA, 1981.
[g] Linden *et al.*, 1979.
[h] McLeese *et al.*, 1980.
[i] Borthwick *et al.*, 1985.
[j] McKenney and Hamaker, 1984.

decisions. Atherinids would be especially useful in addressing environmental questions in specific geographical regions because they are widely distributed and are important in estuarine food webs. Their utility in assessing toxic effects of chemicals during exposures at early life-stages has been described by Goodman *et al.* (1985a,b).

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